these reaggregated tissues contain neurons. Some of the neurons in the whole embryo reaggregates, but very few in the animal cap reaggregates, are GABAergic and glutamatergic. These results suggest that a factor present in the whole embryo is necessary to induce GABAergic and glutamatergic neurons. In a second set of experiments, late blastula and early gastrula animal cap cells were dissociated and cultured until sibling embryos reached swimming tadpole stages. Surprisingly, although consistent with other observations in the literature, these cells do not appear to neutralize; they are negative for the neural markers N-CAM and neural-beta-tubulin as well as mRNAs associated with GABAergic and glutamatergic neurons. However, using immunocytochemistry, these same cells are positive for the GABA (about 30%) and glutamate (about 60%) neurotransmitters. These results suggest that neutralization may require additional signaling and that GABA and glutamate may be serving a different purpose in the cell than currently thought.

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Specification of neurotransmitter phenotypes in *Xenopus laevis*

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Specification of neurotransmitter phenotypes represents a critical step in the development of a functional nervous system. Recent literature indicates that intrinsic factors (transcription factors), lineage, tissue interactions, and environmental factors all play a significant role in this process. In order to identify the state of neurotransmitter specification at various stages in vertebrate development, as well as the factors regulating neuronal phenotype, we have utilized primary cell culture in *Xenopus laevis*. Presumptive neural tissue was removed and dissociated at various stages (gastrula through tailbud) and the cells cultured. Our data show that the percentage of dopamine expressing cells (undifferentiated cells and neural cells) in cell culture decreases from 67% at stage 14 (neural plate) to 44% at stage 18 (neural tube), while the percentage of serotonin-expressing cells in cell culture increases from 5% at stage 14 to 25% at stage 18. Likewise, the percentage of GABAergic cells decreases from 60% at stage 14 to 48% at stage 18. Moreover, the percentage of GABA-positive cells can be altered by treatment with FGF, BMP, and retinoic acid. The data suggest (1) that during early embryogenesis, neural precursors may be co-expressing several different neurotransmitters; and (2) some phenotypes, such as serotonergic, may be induced, while others, such as dopaminergic and GABAergic, are progressively restricted.

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The effects of cell cycle cessation on neurotransmitter specification in *X. laevis*

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A number of genes have been shown to control both the cell cycle and neural phenotype determination, indicating that a relationship exists between the two processes. Studies have also shown that by inducing early cell cycle exit in some systems, early cell fates result. To further examine the connection between the cell cycle and neuron fate determination, this study has examined the effect of early mitosis arrest on the acquisition of neurotransmitter fates. *X. laevis* embryos were treated with DNA synthesis blockers hydroxyurea and aphidicolin (HUA) at various stages of early development to stop cell division until the tailbud stage (Harris and Hartenstein, 1991, *Neuron* 6:499). mRNA probes for neural tubulin and the GABAergic and glutamatergic markers xGAT1 and xVGlut1 were used to observe changes in the CNS and neurotransmitter expression patterns of these embryos induced by HUA. Results show that while neurogenesis and neuronal differentiation occur following premature cell cycle inhibition, which BrdU assays indicate to be incomplete, there are specific changes in neuronal patterning. Neural tubulin expression in HUA-treated embryos closely mirrors that of control embryos in the spinal cord, but it is substantially reduced in the forebrain and midbrain regions of the CNS. xGAT1 and xVGluT1 expression levels are significantly reduced throughout HUA-treated embryos. The changes in these expression patterns are furthermore dependant on the time at which *X. laevis* is treated with HUA. These data suggest the cell cycle plays a role in directing neurogenesis and the neurotransmitter phenotypes of neurons in developing *X. laevis* embryos.

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Molecular profile and developmental potential of migrating neural crest cells

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The neural crest is a stem cell-like population that migrates extensively in embryos and differentiates into a plethora of derivatives ranging from the craniofacial skeleton to ganglia of the peripheral nervous system. Surprisingly, little is known about the molecular pathways that govern the migration and differentiation of neural crest cells. To explore molecular differences in the neural crest as a function of time, particularly related to the mechanisms by which they cease migration and commence gangliogenesis, we screened a macroarrayed library using the purified post-migratory and migratory neural crest cells as probes to identify genes up-regulated at the end of migration. Using in situ hybridization as a secondary screen, we

found many genes enriched in neural crest-derived structures. Post-migratory neural crest genes fell into logical categories including signaling molecules, transcription factors, extracellular matrix molecules and receptors, and cytoskeletal proteins. Although many genes were expressed in other differentiated tissues in addition to the neural crest, a subset were specifically expressed in neural crest-derived structures. These results provide the first molecular profile of genes expressed concomitant with gangliogenesis as well as offering new markers and potential regulatory candidates involved in cessation of migration and onset of differentiation. In addition, we are currently challenging the developmental potential of trunk migrating neural crest cells by heterotopically transplanting them to younger embryos and ask what is the extent of their differentiation abilities.

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Transcription factor AP-2alpha and AP-2gamma act redundantly in zebrafish neural crest specification

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Genetic requirements for neural crest cell specification and survival are poorly defined but relevant to the evolutionary origins of neural crest. Mutagenesis screens have yet to yield a mutant that devoid of neural crest, suggesting that multiple transcription factors expressed in neural crest act redundantly to regulate these events. Expression of activator protein 2 alpha (tfap2a) is an early marker of definitive neural crest in many vertebrates. Mouse and zebrafish *tfap2a* mutants have neural crest but display defects in a subset of neural crest derivatives. Here we show that, as in mouse, zebrafish *tfap2c* is also expressed in premigratory neural crest. Mouse *tfap2c* mutant embryos die from placental defects; we show that wild-type zebrafish embryos injected with *tfap2c* morpholino (MO) have reduced mandibular cartilage but no other gross defects. However, *tfap2a* homozygous mutant embryos injected with *tfap2c* MO lack all neural crest derivatives assayed, including melanophores, sensory neurons, and craniofacial cartilage. Moreover *tfap2a/tfap2c* double knockdown embryos lack expression of markers of premigratory neural crest, and overexpression of *tfap2a* expands the expression of these markers into the neural plate and results in supernumerary melanophores. Mosaic analysis reveals tfap2a/tfap2c role in neural crest development is cell autonomous. These results suggest tfap2 family members function redundantly early in neural crest specification, and that co-option of AP-2 expression to the neural plate border was an essential event in evolution of neural crest.

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A role for anaplastic lymphoma kinase (ALK) function in zebrafish neural crest development

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Anaplastic lymphoma kinase (ALK) is a receptor tyrosine kinase and a member of the insulin receptor superfamily. In humans, constitutively active ALK tyrosine kinase activity, resulting from fusion with nucleophosmin (NPM) to produce an 80-kDa NPM-ALK chimeric oncoprotein, results in anaplastic large cell lymphoma. However, the normal physiological function of ALK has remained mysterious, in part because vertebrate loss of function mutants had not been identified. We recently showed that the zebrafish *shady* locus identified in the Tuebingen 1996 screen encodes a zebrafish ALK orthologue. Homozygotes for strong *shady/alk* alleles completely lack a neural crest-derived pigment cell type, silver iridophores, and die as larvae. These alleles show molecular lesions in *alk* expected to severely disrupt gene function. Thus, we have identified the first in vivo models to study normal ALK functions in vertebrate development and demonstrate a previous unexpected role for Alk in pigment cell development. We are now exploring the precise role of *shady/alk* in zebrafish neural crest development. Whole-amount in situ hybridization with various markers suggest that defects in the iridophore lineage can be detected from the earliest stages of their development. Our data is consistent with a model of Alk function in specification of the iridophore lineage from the neural crest. To test this, we are overexpressing constitutively active ALK (human NPM-ALK) under the control of neural crest specific *Sox10* promoter both in wild type and *shady* embryos.

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Characterization of neural crest and lateral plate mesoderm development in zebrafish hatchback

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Neural crest cells give rise to numerous derivatives including chromatophores, craniofacial cartilages, and neurons and glia of the peripheral nervous system. Likewise, lateral plate mesoderm generates numerous blood, cardiac, and vascular derivatives. The genetic control of neural crest and lateral plate mesoderm development are incompletely understood. *hatchback* (*hbk*) is an ENU-induced recessive embry-